Claims

1. A method for analyzing a single nucleic acid molecule comprising

exposing a single nucleic acid molecule to at least two distinguishable detectable labels for a time sufficient to allow the detectable labels to bind to the single nucleic acid molecule, and

analyzing the single nucleic acid molecule for a coincident event using a single molecule detection system,

wherein the coincidence event indicates that the at least two distinguishable detectable labels are bound to the single nucleic acid molecule.

2. The method of claim 1, wherein the single nucleic acid molecule is denatured to a single stranded form.

3. The method of claim 1, wherein the single nucleic acid molecule is an RNA.

4. The method of claim 1, wherein the single nucleic acid molecule is linearized or stretched prior to analysis.

5. The method of claim 1, wherein the at least two distinguishable detectable labels are present on different unit specific markers.

6. The method of claim 1, wherein the at least two distinguishable detectable labels are present on the same unit specific marker.

7. The method of claim 6, further comprising exposing the single nucleic acid molecule to a third detectable label that binds specifically to a mismatch between the single nucleic acid molecule and the unit specific marker, and wherein a coincident event between the first, second and third detectable labels is indicative of a mismatch.

8. The method of claim 1, further comprising exposing the single nucleic acid molecule and detectable labels to a chemical or enzymatic single stranded cleavage reaction prior to analyzing the single nucleic acid molecule.

9. The method of claim 8, wherein the enzymatic single stranded cleavage reaction uses a single stranded RNA nuclease, a single stranded DNA nuclease, or a combination thereof.

10. The method of claim 9, wherein the single stranded RNA nuclease is RNase I.

11. The method of claim 9, wherein the single stranded DNA nuclease is S1 nuclease.

12. The method of claim 1, further comprising a column purification step.

13. The method of claim 1, wherein the coincident event is a color coincident event.

14. The method of claim 1, wherein one detectable label is attached to a unit specific marker.

15. The method of claim 14, further comprising exposing the single nucleic acid molecule to the labeled unit specific marker in the presence of a polymerase and labeled nucleotides, provided the unit specific marker and nucleotides are differentially labeled.

16. The method of claim 15, wherein a new nucleic acid molecule is formed starting at the unit specific marker and is complementary to the single nucleic acid molecule.

17. The method of claim 16, wherein the new nucleic acid molecule has a signal intensity proportional to its length, and wherein the method is a method of determining integrity of the single nucleic acid molecule.

18. The method of claim 15, wherein the unit specific marker and nucleotides are labeled with a FRET fluorophore pair.

19. The method of claim 1, wherein one detectable label is attached to a unit specific marker and is a first FRET fluorophore, and the other detectable label is incorporated into a newly synthesized nucleic acid molecule hybridized to the single nucleic acid molecule and is the donor or acceptor of the first FRET fluorophore.

20. The method of claim 15, wherein the polymerase is a DNA polymerase.

21. The method of claim 15, wherein the polymerase is a reverse transcriptase.

22. The method of claim 1, wherein the single nucleic acid molecule is present in a nanoliter volume.

23. The method of claim 1, wherein the single nucleic acid molecule is present in at a frequency of 1 in 1,000,000 molecules in an RNA sample.

24. The method of claim 1, wherein the coincident event is the proximal binding of a first detectable label that is a donor FRET fluorophore and a second detectable label that is an acceptor FRET fluorophore, and wherein a positive signal is a signal from the acceptor FRET fluorophore upon laser excitation of the donor FRET fluorophore.

25. The method of claim 24, wherein the single molecule detection system comprises one detector and one laser.

26. The method of claim 1, wherein the detectable labels are present on a unit specific marker that is a DNA, RNA, PNA, LNA or a combination thereof.

27. The method of claim 5, further comprising exposing the nucleic acid molecule to a ligase prior to analysis using the single molecule detection system.

28. The method of claim 1, wherein unbound detectable labels are not removed prior to analysis using the single molecule detection system.

29. The method of claim 1, wherein the detectable labels are provided as molecular beacon probes.

30. The method of claim 1, wherein at least one detectable label is attached to a nucleic acid molecule hybridized to a universal linker attached to a unit specific marker.

31. A composition comprising

a unit specific marker attached to a universal linker that is hybridized to a complementary nucleotide sequence attached to a detectable label.

32. A method for characterizing a polymer sample, comprising

contacting the polymer sample with a plurality of unit specific markers, each of the plurality having a unique and distinct label,

wherein, when bound to the polymer, individual unit specific markers are spaced apart on the polymer such that, if the labels were not distinct from each other, they would be separated by a distance less than the detection resolution.

33. The method of claim 32, wherein the polymer is a nucleic acid molecule.

34. The method of claim 33, wherein the nucleic acid molecule is free-flowing.

35. The method of claim 33, wherein the nucleic acid molecule is fixed to a solid support.

36. The method of claim 33, wherein the nucleic acid molecule is imaged directly.

37. The method of claim 32, wherein the unique and distinct labels are substrates for an enzymatic reaction.

38. The method of claim 37, wherein the enzymatic reaction is selected from the group consisting of a primer extension reaction and a ligase-mediated reaction.

39. The method of claim 33, wherein the nucleic acid molecule is analyzed using a Gene Engine system.

40. The method of claim 32, wherein the polymer is not pre-amplified.

41. The method of claim 32, wherein the polymer is single stranded.

42. The method of claim 37, wherein the enzymatic reaction produces a detectable product.

43. The method of claim 42, wherein the detectable product is not amplified.

44. The method of claim 32, wherein the polymer is detected using a backbone specific label.

45. A method for characterizing a polymer, comprising

fixing the polymer to a solid support,

contacting the polymer sample with a plurality of unit specific markers, each of the plurality having a unique and distinct label, and

determining a pattern of binding of the plurality of unit specific markers to the polymer,

wherein, when bound to the polymer, individual unit specific markers are spaced apart on the polymer such that, if the labels were not distinct from each other, they would be separated by a distance less than the detection resolution.

46. The method of claim 45, wherein the polymer is a nucleic acid molecule.

47. The method of claim 46, wherein the nucleic acid molecule is denatured to a single-stranded form.

48. The method of claim 45, wherein the labels are substrates for enzyme reactions.

49. The method of claim 48, wherein the enzyme reactions produces a detectable product.

50. The method of claim 49, wherein the presence of a detectable product is determined using a single molecule detection system.

51. The method of claim 45, wherein the presence of a detectable product indicates the pattern of binding of the plurality of unit specific markers to the polymer.

52. The method of claim 49, wherein the detectable product is not amplified.

53. The method of claim 45, wherein the polymer is detected using a backbone specific label.

54. The method of claim 45, wherein the polymer is fixed to the solid support in a random orientation.

55. The method of claim 45, wherein the polymer is fixed to the solid support in a non-continuous manner.

56. The method of claim 45, wherein the polymer is characterized by the presence of single nucleotide polymorphisms, microsatellites, insertions, or deletions.

57. The method of claim 45, wherein the unique and distinct labels are differential intensity fluorescent tags.

58. A method for characterizing a polymer sample, comprising

contacting the polymer sample with a plurality of unit specific markers, each of the plurality having a label, and

measuring the distance between consecutive unit specific markers bound to a polymer,

wherein the distance between the consecutive unit specific markers is indicative of a particular haplotype of polymer.

59. The method of claim 58, wherein each of the plurality of unit specific markers is labeled with an identical label.

60. The method of claim 58, wherein the labels are differential intensity fluorescent labels.

61. A method for characterizing a polymer, comprising

attaching a plurality of unit specific markers in a spatially defined manner to an array on a solid support,

contacting the plurality of unit specific markers with an unamplified polymer, and

determining a pattern of binding of the unamplified polymer to the plurality of unit specific markers.

62. The method of claim 61, wherein polymer is a nucleic acid molecule.

63. The method of claim 62, wherein the nucleic acid molecule is not amplified.

64. The method of claim 61, wherein the pattern of binding of the polymer to the plurality of unit specific markers indicates a haplotype for a plurality of genetic loci.

65. The method of claim 61, wherein each spatially defined position in the array is occupied by a haplotype specific unit specific marker.

66. The method of claim 61, wherein the specific unit specific marker is specific for a polymorphism.

67. The method of claim 66, wherein the polymorphism is selected from the group consisting of an single nucleotide polymorphism, a deletion, an insertion, and a genomic amplification.

68. The method of claim 61, wherein the polymer is derived from a single somatic cell hybrid.

69. The method of claim 61, wherein the polymer is a homogenous sample of one chromosome allele.

70. The method of claim 61, wherein each spatially defined position in the array is occupied by an allele specific unit specific marker.

71. A method for determining the haplotype of a nucleic acid sample comprising

amplifying nucleic acid molecules in a nucleic acid sample using an allele-specific polymerase chain reaction (PCR) and a set of four primers, and

analyzing the amplified nucleic acid molecules using a single molecule detection system,

wherein each primer in the set of four primers is unique at its 3' end and is labeled with a unique detectable label.

72. The method of claim 71, wherein the nucleic acid sample is in solution.

73. The method of claim 71, wherein the single molecule detection system is a flow system.

74. A method for determining a length of a nucleic acid molecule comprising

labeling a nucleic acid molecule with a detectable label, and

analyzing the labeled nucleic acid molecule using a single molecule detection system,

wherein the single molecule detection system comprises a narrow channel positioned within an excitation beam, and

the labeled nucleic acid molecule is passed through multiple confocal spots and an average intensity of the labeled nucleic acid passing through the multiple confocal spots is determined.

75. A method for determining a length of a nucleic acid molecule comprising

labeling a nucleic acid molecule with a detectable label, and

analyzing the labeled nucleic acid molecule using a single molecule detection system,

wherein the single molecule detection system comprises an excitation volume to diffraction spot ratio of greater than 10, and

the labeled nucleic acid molecule is passed through a diffraction spot and an integrated intensity of the labeled nucleic acid passing through the diffraction spot is determined.

76. A method for determining a length of a nucleic acid molecule comprising

labeling a nucleic acid molecule with a detectable label, and

analyzing the labeled nucleic acid molecule using a single molecule detection system,

wherein the labeled nucleic acid molecule is imaged using a uniform illumination source, and an integrated intensity of the labeled nucleic acid passing through the diffraction spot is determined.

77. The method of claim 45, 75 or 76, further comprising determining a velocity of the labeled nucleic acid passing through the single molecule detection system.

78. The method of claim 77, wherein the velocity of the labeled nucleic acid is determined using multiple confocal illumination spots.

79. The method of claim 74, 75 or 76, wherein the detectable label is covalently conjugated to the nucleic acid molecule.

80. The method of claim 74, 75 or 76, wherein the detectable label is a fluorophore.

81. The method of claim 74, 75 or 76, wherein the nucleic acid molecule is uniformly labeled along its length.

82. A method for determining the gene profile of a cell comprising

contacting a unit specific marker with an unamplified nucleic acid sample from a single cell, and

determining the binding of the unit specific marker to the nucleic acid sample using a single molecule detection system,

wherein binding of the unit specific marker to the nucleic acid sample indicates that the single cell contains a specific nucleic acid molecule.

83. The method of claim 82, wherein the nucleic acid sample is an RNA sample.

84. The method of claim 82, wherein the nucleic acid sample is a cDNA sample.

85. The method of claim 82, wherein the nucleic acid sample is a genomic DNA sample.

86. The method of claim 82, wherein the single cell is a precursor cell.

87. The method of claim 82, wherein the single cell is a stem cell.

88. The method of claim 82, wherein the single cell is selected from the group consisting of a hemopoietic cell, a neural cell, a liver cell, a skin cell, a cord blood cell.

89. The method of claim 82, wherein the single cell is a cancer cell.

90. The method of claim 82, wherein the single cell is an acute leukemia cell or a Reed Sternberg cell.

91. The method of claim 82, wherein the single cell is an embryo cell.

92. The method of claim 82, wherein the unit specific marker hybridizes to an expressed nucleic acid molecule.

93. The method of claim 82, wherein the unit specific marker hybridizes to an RNA molecule.

94. The method of claim 82, wherein the unit specific marker hybridizes to a genomic DNA molecule.

95. The method of claim 82, wherein the unit specific marker is specific for a genetic abnormality.

96. The method of claim 82, wherein the unit specific marker is a plurality of unit specific markers.

97. The method of claim 82, wherein determining the binding of the unit specific marker to the nucleic acid sample comprises determining a pattern of binding of the unit specific marker to the nucleic acid sample.

98. The method of claim 82, wherein the unit specific marker is a unit specific marker that binds to a known nucleic acid molecule.

99. The method of claim 82, further comprising comparing the pattern of binding of the unit specific marker to a second binding pattern.

100. The method of claim 99, wherein the second binding pattern is of a different cell.

101. The method of claim 99, wherein the second binding pattern is of a non-cancerous cell.

102. The method of claim 99, wherein the second binding pattern is of a differentiated cell.

103. The method of claim 82, wherein the unit specific marker is conjugated to a detectable label.

104. The method of claim 103, wherein the detectable label is selected from the group consisting of differential intensity fluorophores, differential lifetime fluorophores, and fluorescence resonance energy transfer (FRET) fluorophores.

105. The method of claim 82, wherein the binding of the unit specific marker to the nucleic acid sample is determined by imaging.

106. The method of claim 82, wherein the binding of the unit specific marker to the nucleic acid sample is determined by confocal detection.

107. A method for quantitating a nucleic acid molecule in a cell comprising

contacting a unit specific marker with an unamplified nucleic acid sample from one or more cells, and

measuring the level of binding of the unit specific marker to the nucleic acid sample using a single molecule detection system,

wherein the unit specific marker is conjugated to a detectable label, and

wherein the level of binding of the unit specific marker to the nucleic acid sample is indicative to the level of the nucleic acid molecule in the sample.

108. A method for determining the presence of a polymorphism in a nucleic acid molecule comprising

allowing a wild type unit specific marker of a specified length to hybridize to a nucleic acid molecule in a nucleic acid sample from one or more cells,

then exposing the nucleic acid sample, after hybridization and washing, to an enzymatic or chemical reaction in order to cleave a heteroduplex at a single stranded region, and

detecting one or more cleavage products of the enzymatic or chemical reaction using a single molecule detection system,

wherein the wild type unit specific marker is labeled at one or both ends with a first detectable label,

the nucleic acid molecule in the nucleic acid sample is labeled at one or both ends with a second detectable label that is distinct from the first detectable label, and

a double stranded cleavage product having both first and second detectable labels and a length of less than the specified length of the wild type unit specific marker is indicative of a polymorphism in the nucleic acid molecule from the nucleic acid sample.

109. The method of claim 108, wherein the nucleic acid sample is an amplified sample and the method detects errors in an amplification process.

110. The method of claim 108, wherein the second detectable label is incorporated into the nucleic acid molecule during the amplification process.

111. The method of claim 108, wherein the enzymatic reaction is a reaction with an enzyme selected from the group consisting of endonuclease VII and RNase.

112. The method of claim 108, wherein the chemical reaction comprises reaction with osmodium tetroxide.

113. The method of claim 108, wherein the nucleic acid molecule is DNA.

114. The method of claim 108, wherein the nucleic acid molecule is RNA.

115. The method of claim 108, wherein the wild type unit specific marker is labeled at its 3' end and the nucleic acid molecule is labeled at its 5' end.

116. The method of claim 108, wherein the wild type unit specific marker is labeled at its 5' end and the nucleic acid molecule is labeled at its 3' end.

117. The method of claim 108, wherein the wild type unit specific marker and the nucleic acid molecule are both labeled at their 3' and 5' ends.

118. The method of claim 108, wherein the detection of the cleavage products is not dependent upon amplification of the cleavage products.

119. A method for determining the presence of a polymorphism in a nucleic acid molecule comprising

amplifying one or more nucleic acid molecules using a first and a second primer to form an amplified nucleic acid sample having amplified nucleic acid molecules of a defined length,

denaturing and re-hybridizing the amplified nucleic acid sample, and

then exposing the re-hybridized, amplified nucleic acid sample to an enzymatic or chemical reaction in order to cleave a heteroduplex at a single stranded region, and

detecting one or more cleavage products of the enzymatic or chemical reaction using a single molecule detection system,

wherein the first primer is labeled with a first detectable label, and the second primer is labeled with a second detectable label distinct from the first detectable label, and

a double stranded cleavage product comprising either the first or the second detectable label and a length of less than the defined length of the amplified nucleic acid molecules is indicative of a polymorphism in an amplified nucleic acid molecule from the amplified nucleic acid sample.

120. The method of claim 119, wherein the re-hybridized, amplified nucleic acid sample is fixed to a solid support prior to the enzymatic or chemical reaction at either or both ends.

121. The method of claim 119, wherein the double stranded cleavage product is fixed on a solid support and imaged.

122. A method for identifying the source of a nucleic acid molecule comprising

digesting a nucleic acid molecule with a first and a second restriction endonuclease to form nucleic acid fragments,

labeling a first end of a nucleic acid fragment with a first detectable label, and labeling a second end of the nucleic acid fragment with a second detectable label that is distinct from the first detectable label to form an end-labeled nucleic acid fragment,

analyzing the end-labeled nucleic acid fragment using a single molecule detection system to detect the first and second detectable label, and determine a length of an end-labeled nucleic acid fragment by measuring a distance between the first and the second detectable labels for each end-labeled nucleic acid fragment,

wherein prior to labeling the first end and the second end of the nucleic acid fragment are different, and

a plurality of lengths of a plurality of end-labeled nucleic acid fragments identifies the source of a nucleic acid molecule.

123. The method of claim 122, wherein the first end and the second end of the nucleic acid fragment are selected from the group consisting of a 3' overhang, a 5' overhang, and a blunt end.

124. The method of claim 122, wherein the first and second detectable labels are conjugated to the nucleic acid fragments indirectly.

125. The method of claim 122, wherein the first and second detectable labels are conjugated to the nucleic acid fragments using a polymerase reaction.

126. The method of claim 125, wherein the polymerase reaction comprises an additional primer.

127. The method of claim 122, wherein one or both the first and second restriction endonucleases are chimeric.

128. The method of claim 122, wherein the nucleic acid molecule is unamplified.

129. The method of claim 122, wherein the nucleic acid molecule is a bacterial artificial chromosome (BAC).

130. The method of claim 122, wherein the nucleic acid molecule is a yeast artificial chromosome (YAC).

131. The method of claim 122, wherein the nucleic acid molecule is from a forensic sample.

132. The method of claim 122, wherein the nucleic acid molecule is from a sample intended for paternity determination.

133. The method of claim 122, wherein the nucleic acid molecule is labeled with a non-specific backbone label.

134. The method of claim 122, wherein the nucleic acid fragment is labeled with a non-specific backbone label.

135. A method for identifying the source of a nucleic acid molecule comprising

digesting a nucleic acid molecule with a first restriction endonuclease to form nucleic acid fragments,

labeling nucleic acid fragments with a non-specific backbone label,

analyzing the labeled nucleic acid fragments using a single molecule detection system, and

determining a length of the labeled nucleic acid fragment by measuring a time between the first detected non-specific backbone label and the last detected non-specific backbone label for each end-labeled nucleic acid fragment,

wherein, prior to labeling, the first end and the second end of the nucleic acid fragment are different, and

a plurality of lengths of a plurality of end-labeled nucleic acid fragments identifies the source of a nucleic acid molecule.

136. The method of claim 135, wherein the first end and the second end of the nucleic acid fragment are selected from the group consisting of a 3' overhang, a 5' overhang, and a blunt end.

137. A method for detecting a mutant polymer comprising

contacting a polymer with one or a plurality of unique and distinct mutant-specific unit specific markers each labeled with a first detectable label,

contacting the polymer with a polymer-specific unit specific marker that binds to wild type and mutant polymers and is labeled with a second detectable label, and

analyzing the polymer for coincidence binding of the presence of the first and second detectable labels on the polymer,

wherein the first and second detectable labels are unique and distinct, and the coincident binding indicates the polymer is a mutant polymer.

138. The method of claim 137, wherein the polymer is a nucleic acid.

139. The method of claim 138, wherein the nucleic acid is a DNA or RNA.

140. The method of claim 137, wherein the coincident event is detected using a single molecule detection system.

141. The method of claim 137, wherein the mutant-specific unit specific markers are specific for a single nucleotide polymorphism, a deletion, an insertion, a genomic amplification, or an inversion.

142. The method of claim 137, wherein the first and second detectable labels are of the same type.

143. The method of claim 137, wherein the first and second detectable labels are independently selected from the group consisting of an electron spin resonance molecule, an electrical charge transferring molecule, a fluorescent molecule, a chemiluminescent

molecule, a radioisotope, an enzyme, an enzyme substrate, a chromogenic substrate, a biotin molecule, a streptavidin molecule, a semiconductor nanocrystal, a semiconductor nanoparticle, a colloid gold nanocrystal, a ligand, a microbead, a magnetic bead, a paramagnetic particle, a quantum dot, an affinity molecule, a protein, a peptide, nucleic acid, a carbohydrate, an antigen, a hapten, an antibody, an antibody fragment and a lipid.

144. The method of claim 137, wherein the first and second detectable labels are fluorescent molecules.

145. The method of claim 137, wherein the polymer is free-flowing.

146. The method of claim 137, wherein the polymer is fixed to a solid support.

147. The method of claim 137, wherein the polymer is imaged directly.

148. The method of claim 137, wherein the coincident binding is detected by the coincident direct detection of the first and second detectable labels.

149. The method of claim 137, wherein the coincident binding is a proximal binding of the first detectable label that is a donor FRET fluorophore and the second detectable label that is an acceptor FRET fluorophore, and is detected by a signal from the acceptor FRET fluorophore upon laser excitation of the donor FRET fluorophore.

150. The method of claim 137, wherein the coincident binding is a proximal binding of the second detectable label that is a donor FRET fluorophore and the first detectable label that is an acceptor FRET fluorophore, and is detected by a signal from the acceptor FRET fluorophore upon laser excitation of the donor FRET fluorophore.

151. The method of claim 140, wherein the single molecule detection system is a Gene Engine™ system.

152. The method of claim 137, wherein the polymer is not pre-amplified.

153. The method of claim 137, wherein the polymer is single stranded.

154. The method of claim 137, wherein the plurality of mutant-specific unit specific markers is at least 3, at least 4, at least 5, at least 10, at least 15, at least 20, at least 25, at least 50, at least 75 or at least 100.

155. The method of claim 137, wherein the mutant-specific unit specific markers bind to known mutant sequences.

156. The method of claim 137, wherein the mutant polymer indicates a predisposition to or presence of a disease.

157. The method of claim 156, wherein the disease is cancer.

158. The method of claim 156, wherein the disease is cystic fibrosis.

159. The method of claim 157, wherein the cancer is colorectal cancer.

160. The method of claim 137, wherein the polymer is present in a bodily sample.

161. The method of claim 139, wherein the DNA is genomic nuclear DNA, mitochondrial DNA or cDNA.

162. The method of claim 139, wherein the RNA is mRNA.

163. The method of claim 137, wherein the polymer is condensed.

164. The method of claim 137, further comprising a column purification step.

165. The method of claim 137, wherein the coincident event is a color coincident event.

166. The method of claim 137, wherein the polymer is present in a nanoliter volume.

167. The method of claim 137, wherein the polymer is present in at a frequency of 1 in 1,000,000 molecules in a sample.

168. The method of claim 137, wherein the unit specific markers are comprised of DNA, RNA, PNA, LNA or a combination thereof.

169. The method of claim 137, wherein unbound detectable labels are not removed prior to analysis using the single molecule detection system.

170. The method of claim 137, wherein the first and second detectable labels are provided as molecular beacon probes.

171. The method of claim 137, wherein at least the first or the second detectable label is attached to a nucleic acid molecule hybridized to a universal linker attached to a unit specific marker.

172. The method of claim 146, wherein the polymer is fixed to the solid support in a random orientation.

173. The method of claim 146, wherein the polymer is fixed to the solid support in a non-continuous manner.

174. The method of claim 137, wherein the binding of the mutant-specific unit specific marker and the polymer-specific unit specific marker to the polymer is determined by confocal detection.

175. The method of claim 137, wherein detection of coincident binding of both the polymer-specific unit specific marker and any one or more of the mutant-specific unit specific markers indicates the polymer is a mutant polymer.